Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(1):20-28
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Related substances of azilsartan medoxomil: Synthesis and characterization

Madhuresh K. Sethi, Vijendra Singh Rawat, Jayaprakash Thirunavukarasu, Raja Krishna Yeramalla and Anish Kumar

R & D, Mylan Laboratories Ltd., Plot No. 31, 32, 33 and 34 A ANRICH Industrial Estate, Bollaram (Village), Jinnaram (Mandal), Medak (Dt), Andhra Pradesh, India

---

ABSTRACT

AzilsartanMedoxomil is the prodrug 2-ethoxy-1-([2'-(5- oxo 4, 5 – dihydro - 1, 2, 4-oxadiazol-3-yl) biphenyl-4-yl]methyl) 1H-benimidazole-7-carboxylic acid which is used as a antihypertensive drug. During the process optimization of Azilsartanmedoxomil novel related substances (impurities) were observed. These related substances were prepared and characterized by MASS, NMR and HPLC.

Keywords: Related substances, Azilsartan, Antihypertensive, synthesis

---

INTRODUCTION

AzilsartanMedoxomil Potassium is chemically named as (5-Methyl-2-oxo-1, 3-dioxol-4yl) methyl 2-ethoxy-1-{[2-(5-oxo-4, 5-dihydro-1, 2, 4-oxadiazol-3-yl) biphenyl-4-yl]methyl}- 1H-benzimidazole-7-carboxylatemonopotassium salt. Azilsartanmedoxomil is the prodrug of 2-ethoxy-1-([2'-(5-oxo4,5 – dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl) 1H-benimidazole-7-carboxylic acid.It is a white crystalline powder insoluble in water, slightly soluble in solvents such as acetone, and acetonitrile, freely soluble in methanol, dimethylsulfoxide, and dimethylformamide, soluble in solvents such as acetic acid, and very slightly soluble in solvents tetrahydrofuran and 1-octanol. The US Food and Drug Administration (FDA) has approved Edarbi tablet (AzilsartanMedoxomil Potassium) on February 25, 2011, to treat hypertension in adults. It is available in 80mg and 40 mg dosages, with the recommended dosage set at 80mg once in a day [1].Angiotensin II hormone plays a vital role in activation of renin-angiotensin-aldosterone systems well as in regulation of blood pressure, fluid-electrolyte balance, and also in pathophysiology of hypertension. Activation of type 1 angiotensin receptor which is a member of G protein coupled receptor efficiently controls the numerous effects of AII which are vasoconstriction, secretion of aldosterone and vasopressin and cellular proliferation. So blocking of AII receptor will also block receptor-1, and it will lead to termination of the whole course of action mentioned above; so all blocker will be helpful in the management of cardiovascular and renal diseases as therapeutic agent.The active moiety of AMP is revealed by hydrolysis of the medoxomil ester and it converts into azilsartan which is an active angiotensin II receptor blocker and more effective in lowering blood pressure within 24 hours as compared to valsartan and olmesartan[2-5].There are several methods that are reported for preparation of azilsartan [6-15].The presence of related substances in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug products. Therefore, it is necessary to study the impurity (related substance) profile of the API to be used in the manufacturing of the drug product. International Conference on Harmonization (ICH)guidelines recommends identifying and characterizing all related substances

that are present at level less than 0.10% [16].In this context a comprehensive study was undertaken to synthesize and characterize a total of 13 related substancesof Azilsartan.

MATERIALS AND METHODS

Analytical

HPLC was carried out using Waters HPLC having 2487 UV detector with empower chromatography software. Column symmetry used is C18, 158× 4.6 mm, 5 µm with UV- Detector at 250 nm. Flow rate is maintained at 1.0 ml/min with injection volume of 10 µL using diluent Acetonitrile. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSOon a Bruker Advance 300 spectrometer. The chemical shifts are reported in δ ppm relative to TMS (δ 0.00) and DMSO and D_2Oas internal standards respectively. Electron Spray Ionization-Mass spectra (ESI-MS) of isolated compounds were measured using Agilent 1100 LC/MSD Trap SL instrument.

Chemicals

All the chemicals used were of commercial grade

Synthesis

Preparation of Related substance A

5 % potassium hydroxide (20 ml) was added to 1-[(2´-cyanobiphenyl-4-yl) methyl]-2-ethoxy benzimidazole -7-carboxylate (5 g) in methanol (100 ml).The mixture was stirred at room temperature for 3 h. After the completion of reaction as indicated by TLC, the reaction mass was distilled under vacuum at 50°C to remove methanol. The distilled reaction mass was diluted with water and then acidified with dilute hydrochloric acid. The resulting suspension was filtered, and dried to obtain related substance**A** (4.4 g, 90.7 % Yield). HPLC purity – 95 %. ^{1}H NMR (300 MHz, DMSO–d_6) 13.0 (1H, s), 7.95- 7.94 (1H, m), 7.92-7.70 (2H, m), 7.68-7.58 (5H, m), 7.56 – 7.53 (3H, m), 7.16 (2H, s), 4.65-4.58 (2H, m), 1.43-1.38 (3H, m); Mass (*m/z*) = 398.1 (M+ 1)

Preparation of Related substance B

A mixture of hydroxyl amine hydrochloride (15.5 g, 0.223mol) and sodium bi carbonate (27 g, 0.308 mol) in ethanol (150 ml) was stirred at room temperature for 60 min. The reaction mixture was maintained at 50 °C for another 60 min and then 1-[(2´cyanobiphenyl-4-yl) methyl]-2-ethoxy benzimidazole -7-carboxylate (4.9 g, 0.012 mol) was added. The reMixture maintained at 50-60°C for 5 h. After completion of reaction, reaction mass was quenched in water at below 15° C. The resulting suspension solids was filtered and washed with water. The solids were dried under vacuum at 60 °C to get compound **B** (3.2 g, 60 % Yield). HPLC purity = 96.5 %. ^{1}H NMR (300 MHz, DMSO –d_6) 1.39 – 1.44 (3H, m), 4.64 - 4.57 (2H, m), 5.67-5.65 (2H, m), 6.98 - 6.99 (3H, m), 7.18 – 7.43 (11H, m), 12.5 (1H, s), Mass (*m/z*) = 431.1 (M+H)

Preparation of Related substance C

A mixture of AZP amidoximerelated substance(2 g, 0.0046 mol), chloromethyl-5-methyl-1, 3-dioxol-2-one (1 g, 0.0067 mol), potassium carbonate (0.9 g, 0.0065 mol) in dimethyl formamide (100 ml) was stirred at room temperature for about 3 h.After the completion of reaction, the reaction mass quenched in water and stirred about an hour. The resulting suspension was extracted with methylene dichloride by evaporation to obtain an oil and thereafter purified by column chromatography (methylene dichloride/methanol as eluent) to provide the title compound **C(**0.36g, 14.3 % yield). HPLC purity = 95.6 %, ^{1}H NMR (300 MHz, DMSO –d_6) 2.01-2.03 (1H, m), 2.11- 2.04 (3H, m), 2.19-2.14 (3H, m), 4.97 – 4.65 (3H, m), 5.71-5.63 (2H, m), 6.97 – 6.93 (1H, m), 7.12 – 6.99 (2H, m), 7.38 – 7.22 (11H, m),Mass (*m/z*) = 543.2 (M+H)

Preparation of Related substanceD

A mixture of 1-[(2´cyanobiphenyl-4-yl)methyl]-2-ethoxy benzimidazole -7-carboxylic acid (20 g, 0.050 mol), potassium carbonate(10.4 g, 0.075 mol) and potassium iodide (2.0 g, 0.012 mol) in dimethyl formamide (100 ml) was stirred at room temperature for 2 h. 4-Chloromethyl -5-methyl-1, 3-doixol-2-one (11.0 g, 0.074 mol) dissolved in DMF was added slowly to the mixture. The reaction mixture was stirred at room temperature for 5 h. After the completion of reaction the reaction mass was quenched with water and extracted with dichloromethane and concentrated the organic layer to get the compound **D** (24.0 g, 93 % Yield). HPLC purity = 97 %, ^{1}H NMR (300 MHz, DMSO –d_6) 1.52- 1.47 (3H, m), 2.14 (3H, s), 4.71- 4.64 (2H, m), 4.91 (2H, s), 5.71 (2H, s), 7.43-7.4 (2H, m), 7.48- 7.45 (1H, m), 7.59 - 7.48 (4H, m), 7.60 – 7.59 (2H, m), 7.61 – 7.60 (2H, m), Mass (*m/z*) = 510.1 (M+H)

Preparation of Related substance E
A mixture of hydroxyl amine hydrochloride (16 g, 0.2302 mol) in ethanol (180 ml) was stirred at room temperature for 60 min Sodium carbonate (18.7 g, 0.1764 mol) was added slowly over a period of 60 minutes to the above mixture. The obtained mixture was maintained at 50 °C for another 60 minutes. To the resulting mixture 1-[(2´cyanobiphenyl-4-yl) methyl]-2-ethoxy benzimidazole -7-carboxylate (5 g, 0.0121 mol) was added. Reaction temperature was raised to 70-80º C and maintained for 5 h. Water was added slowly to reaction mixture at temperature below 15 ºC. The solid obtained was filtered and washed with water. The solid was dried under vacuum at 60 ºC to get compound **E**(4.1 g, 78.0 % Yield). HPLC purity = 96 %, ^{1}H NMR (300 MHz, DMSO –d_6) 1.44 – 1.39 (3H, m), 3.70 (3H, s), 4.65 – 4.58 (2H, m), 5.51 (2H, s), 7.28- 7.27 (2H, m), 7.34 – 7.31 (1H, m), 7.39 – 7.34 (8H, m), 7.41 (1H, s), 7.44 – 7.41 (1H, d), Mass (*m/z*) = 430.2 (M+H)

Preparation of Related substance F
10 % Sodium hydroxide (200 ml) was added to AZP amide (20 g, 0.046 mol) in methanol (800 ml).The mixture stirred at room temperature for three hours. After the completion of reaction, the reaction mass distilled under vacuum at 50°C to remove methanol. The distilled reaction mass was diluted with water and the mixture was acidified with diluteHCl. The resulting suspension was filtered and washed with isopropyl ether and dried to get compound **F** (15.56 g, 80.5 % Yield). HPLC purity = 97.19 %, ^{1}H NMR (300 MHz, DMSO –d_6) 1.43 – 1.39 (3H, m), 4.64- 4.57 (2H, m), 5.66 (2H, m), 7.01- 6.99 (2H, m), 7.17 – 7.15 (1H, m), 7.43 – 7.20 (7H, m), 7.52 – 7.46 (1H, m), 7.68 – 7.66 (2H, m), 13.19 (1H, s), Mass (*m/z*) = 416.1 (M+H)

Preparation of Related substanceG
A mixture of AZP acid amide related substance (1 g, 0.002 mol), chloromethyl -5-methyl-1, 3-doixol-2-one (1 g, 0.006 mol),potassium carbonate (0.5 g, 0.003),potassium iodide (0.1g, 0.0006) in DMF (100 ml) was stirred at room temperature for about three hours.After the completion of reaction,the reaction mass was quenched in water and stirred for about an hour. The resulting suspension was filtered, washed with water and dried to obtainrelated substance G. The material was dried atmospherically to get the compound **G**(1 g, 78.7 % Yield). HPLC purity = 95.8 %, ^{1}H NMR (300 MHz, DMSO –d_6) 2.02-2.04 (1H, m), 2.10- 2.04 (3H, m), 2.17-2.13 (3H, m), 4.95 – 4.63 (3H, m), 5.72-5.62 (2H, m), 7.14 – 6.97 (2H, m), 7.39 – 7.25 (11H, m), Mass (*m/z*) = 528.2 (M+H)

Preparation of Related substanceH
20 % Sodium hydroxide (70 ml) was added to Methyl 2-ethoxy benzimidazole - 7 - carboxylate (5 g, 0.0227 mol) and stirred at room temperature for three hours. After the completion of reaction, charge acetone 25 ml and acidified with diluteHCl. The resulting suspension was filtered, washed with water and dried to obtain compound **H**(4.42 g, 93.6 % Yield). HPLC purity = 97.81 %, ^{1}H NMR (300 MHz, DMSO – d_6) 1.42 – 1.37 (3H, m), 4.55 – 4.48 (2H, m), 7.17 – 7.11 (1H, m), 7.62 – 7.59 (2H, m), 12.0 (1H, s), 13.0 (1H, s), Mass (*m/z*) = 221.1 (M+H)

Preparation of Related substanceI
Methyl 2-ethoxy[(2´-(hydroxyamidino) biphenyl-4-yl) Methyl]-1H-benzimidazole-7-carboxylate carboxylate (10 g, 0.022 mole) was dissolved in acetone (100 ml) at 25-30 ° C and concentrated hydrochloric acid was added. The resulting reaction mixture was refluxed at 56 °C for 3 h.The mixture was cooled to room temperature, filtered and washed with acetone. The material was finally dried at 50 ° C to get compound **I**. (9.1 g, 97 % Yield). HPLC purity = 97.0 %, ^{1}H NMR (300 MHz, DMSO – d_6) 3.64 (3H, s), 5.3 (2H, m), 7.11 – 7.06 (3H, m), 7.32 – 7.25 (4H, m), 7.58 – 7.55 (3H, m), 7.70-7.68 (1H, m), 9.02- 8.87 (1H, s), 11.09 (1H, s), 11.56 (1H, s), 12.64 (1H, s), Mass (*m/z*) = 207.2 (M+H)

Preparation of Related substanceJ
Methyl 1-[[2´-(4,5-dihydro-5-oxo-4H-1,2,4-oxadiazol-3-yl) biphenyl-4-yl) Methyl]-2-ethoxy-1H- benzimidazole-7-carboxylate (10 g, 0.021 mole) was dissolved in acetone (100 ml) at 25-30 ° C and concentrated hydrochloric acid (20 ml) was added. The resulting reaction mixture was refluxed at 56 °C for 3 h.The mixture was cooled to room temperature, filtered and washed with acetone. The material was dried at 50 °C to get compound **J**. (8.2 g, 88.2 % Yield). HPLC-99.18 %, ^{1}H NMR (300 MHz, DMSO – d_6) 3.61 (3H, s), 5.28 (2H, m), 7.10 – 7.02 (3H, m), 7.27-7.23 (4H, m), 7.48 – 7.45 (1H, m), 7.57 – 7.52 (1H, m), 7.7 – 7.64 (2H, m), 11.55 (1H, s), 12.47 (1H, s), Mass (*m/z*) = 416.2

Preparation of Related substanceK

Benzyl bromide (7.27 g, 0.042 mol) was added to a stirred mixture of methyl 1-[[2´-(4,5-dihydro-5-oxo-4H-1, 2, 4-oxadiazol-3-yl) biphenyl-4-yl) Methyl]-2-ethoxy-1H- benzimidazole-7-carboxylate (5 g, 0.010 mole) and sodium carbonate (6.76 g, 0.063 mol) in dimethyl sulfoxide (50 ml). The reaction mixture was stirred for overnight at 25-30°C. Inorganic salts were removed by adding methylene chloride (50 mL) and water (20 mL). The desired compound was extracted from methylene chloride by evaporation to obtain an oil. Oily material was crystalized in isopropyl ether to get compound **K** (4.5 g, 75.6 % Yield). HPLC purity = 98.1 %, ^{1}H NMR (300 MHz, DMSO – d_6) 1.52 – 1.45 (3H, m), 3.69 (3H, s), 4.70 – 4.63 (2H, m), 5.12 (2H, s), 5.61 (2H, s), 7.55 – 6.60 (16H, m), Mass (*m/z*) = 561.6 (M+H)

Preparation of Related substanceL

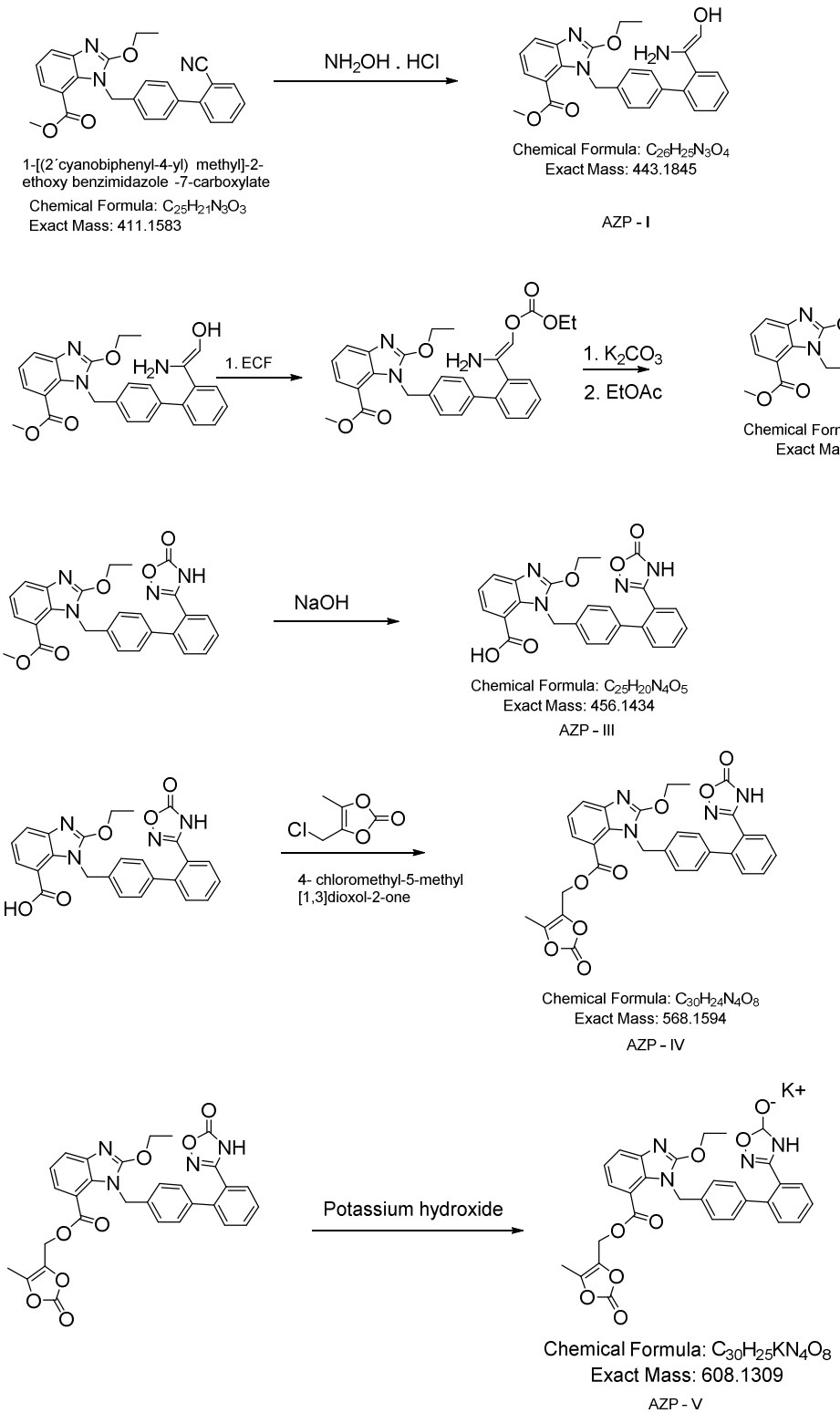
2-methyl -4-cyano biphenyl (5 g, 0.0258 mol) was added to a stirred mixture of hydroxyl amine hydrochloride (27 g, 0.3884 mol) and sodium carbonate (27.4 g, 0.2585 mol) in dimethyl sulfoxide 125 ml. The reaction mixture was stirred at 85°C for 15 h. Inorganic salts were removed by adding methylene chloride (50 mL) and water (20 mL). The desired compound wasextracted from methylene chloride by evaporation to obtain an oil as product **L**(4.0 g, 68.4 % Yield). HPLC purity = 97.1 %, ^{1}H NMR (300 MHz, DMSO – d_6) 1.52 – 1.45 (3H, m), 3.75 (3H, s), 4.70 – 4.63 (2H, m), 5.61 (2H, s), 7.55 – 6.60 (12H, m), Mass (*m/z*) = 227.1 (M+H)

Preparation of Related substanceM

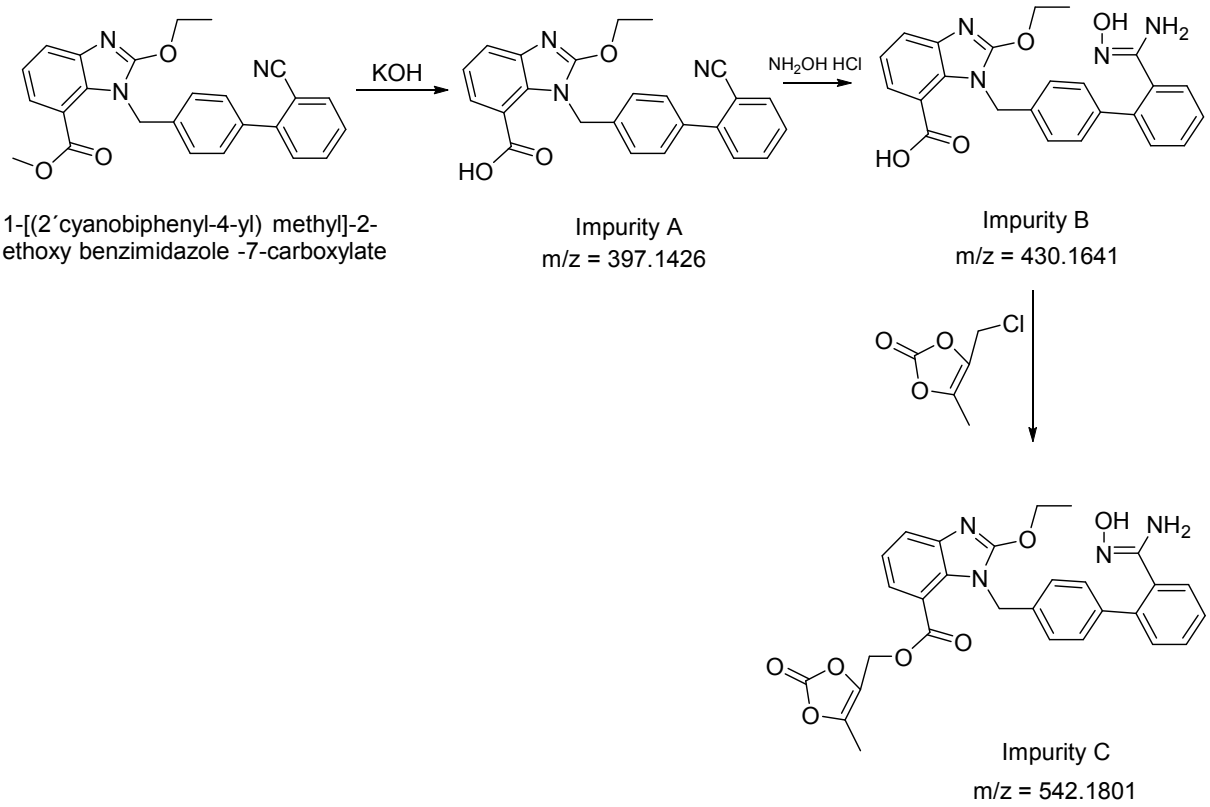
Methyl biphenyl oxime (4 g, 0.0176 mol) was dissolved with methylene dichloride (40 ml) in presence of triethylamine (2.68 g, 0.0264). Ethylchloroformate (2.5 g, 0.0230 mol) was added slowly to reaction mixture at 0-5°C.The reaction mixture was stirred at same temperature for 3 h. The reaction mixture was washed with water and concentrated under vacuum to get oily product. Oily product obtained was dissolved in ethyl acetate at 25-30°C. Potassium carbonate was added to reaction mixture and stirred at 77-79 °C for 12 h. It was then cooled to 25-35 °C, filtered and washed with ethyl acetate and water and dried under vacuum at 40 °C to get compound **M**(2.58 g, 58.1 % Yield). HPLC purity = 98.2 %, ^{1}H NMR (300 MHz, DMSO – d_6) 3.15 (3H, s), 7.15 – 7.10 (3H, m), 7.60 – 7.55 (4H, m), 12.37 (1H, s),Mass (*m/z*) = 253.1 (M+H)

RESULTS AND DISCUSSION

Synthetic route followed for preparation of AzilsartanMedoximil has been given in Scheme 1. The preparation of Azilsartan involves reaction of hydroxyl amine hydrochloride with 1-[(2´-cyanobiphenyl-4-yl) methyl]-2-ethoxy benzimidazole -7-carboxylate using sodium bicarbonate base in DMSO solvent to give methyl 2-ethoxy [(2´-(hydroxyamidino) biphenyl-4-yl) Methyl]-1H- benzimidazole-7-carboxylate (AZP-1).AZP – I is then reacted with ethyl chloroformate and triethyl amine to give methyl 1-[[2´-(4, 5-dihydro-5-oxo-4H-1, 2, 4-oxadiazol-3-yl) biphenyl-4-yl) Methyl]-2-ethoxy-1H- benzimidazole-7-carboxylate (AZP-II). AZP – II thus obtained is then hydrolyzed using sodium hydroxide solution to give AZP – III. AZP –III is reacted with chloromethyl-5-methyl-1, 3-dioxol-2-one in presence of DBU and finally with potassium ethyl hexanoate to give Azilsartanmedoxomil.

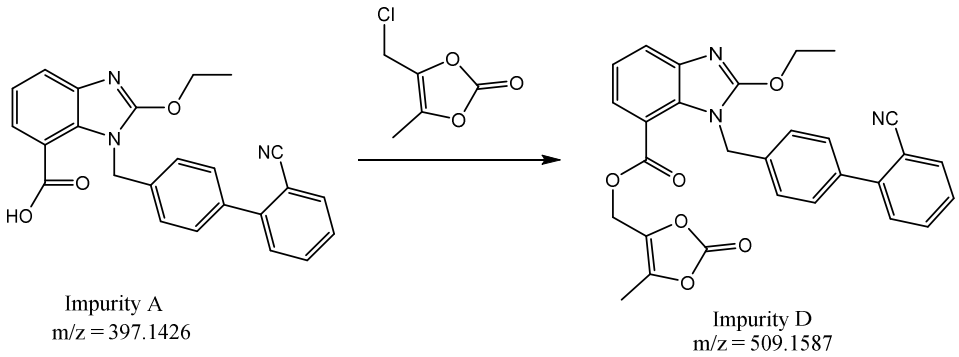


Scheme 1. Synthetic route followed for preparation of Azilsartanmedoximil
Pathway for Series 1 *Related substances* (*Related substances* A to C)



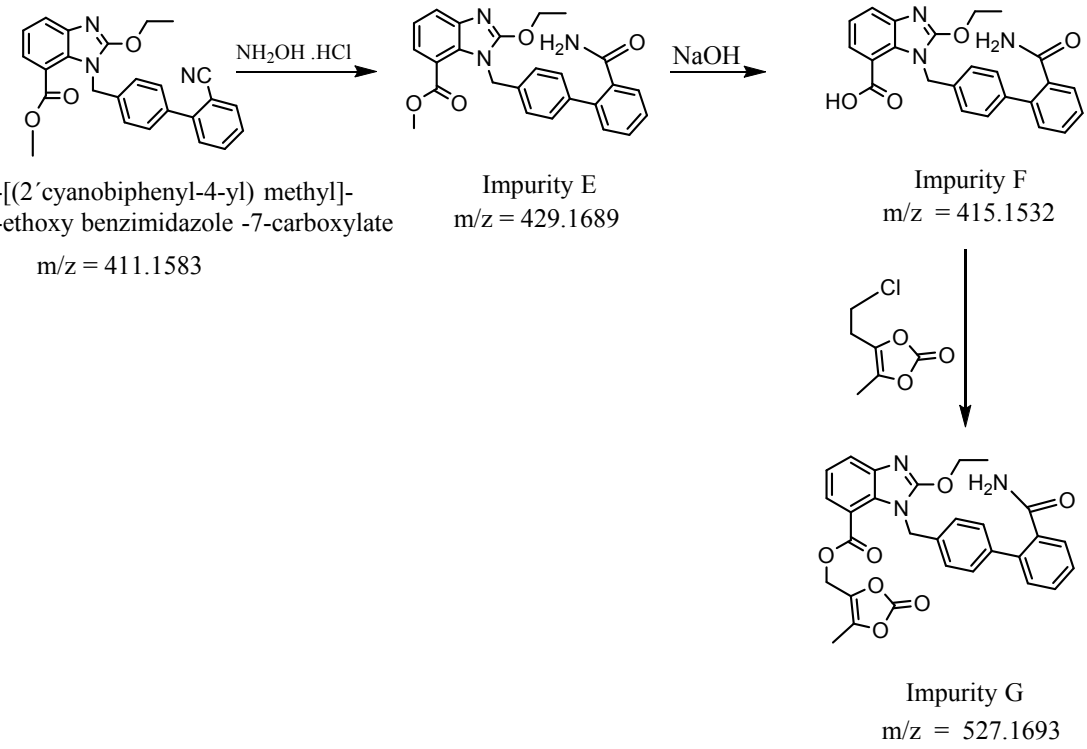
Related substance A could be formed by basic hydrolysis of 1-[(2´-cyanobiphenyl-4-yl) methyl]-2-ethoxy benzimidazole -7-carboxylate. *Related substance* A could then react with hydroxyl amine hydrochloride in presence of sodium bicarbonate to give *related substance* B which in turn could react with chloromethyl-5-methyl-1, 3-dioxol-2-one to give finally *related substance* C.

Pathway for Series 2 *Related substance*(*Related substance* D)



Related substance A formed by basic hydrolysis of 1-[(2´cyanobiphenyl-4-yl) methyl]-2-ethoxy benzimidazole -7-carboxylate could react directly with chloromethyl-5-methyl-1, 3-dioxol-2-one to give related substance D

Pathway for Series 3 *Related substance*s (*Related substance* E to *Related substance* G)



1-[(2´cyanobiphenyl-4-yl) methyl]-2-ethoxy benzimidazole -7-carboxylate

m/z = 411.1583

Impurity E

m/z = 429.1689

Impurity F

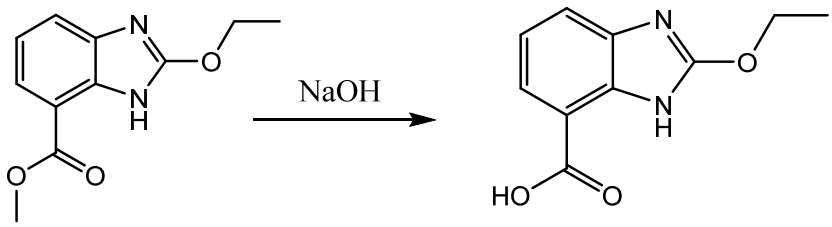
m/z = 415.1532

Impurity G

m/z = 527.1693

1-[(2´cyanobiphenyl-4-yl) methyl]-2-ethoxy benzimidazole- 7 - carboxylate could get partially hydrolyzed to get amide related substance E, which could undergo further basic hydrolysis to get related substance F. Related substance F could then finally react with chloromethyl-5-methyl-1, 3-dioxol-2-one to get related substance G.

Pathway for Series 4*Related substance* (*Related substance* H)



Methyl 2-ethoxy benzimidazole - 7 - carboxylate

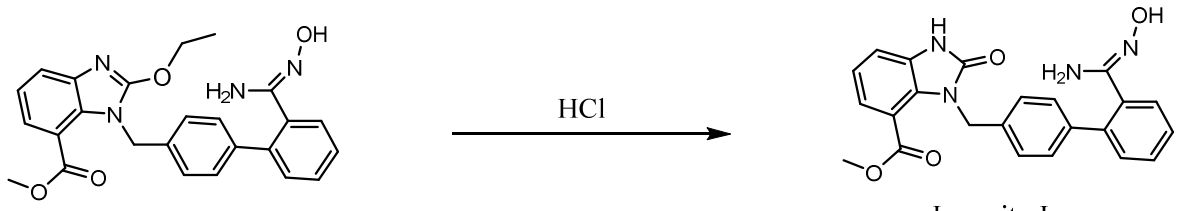
m/z = 220.0848

Impurity H

m/z = 206.0691

Related substance H could be prepared by basic hydrolysis of Methyl 2-ethoxy benzimidazole - 7 - carboxylate.

Pathway for Series 5 *Related substance*(Related substance I)



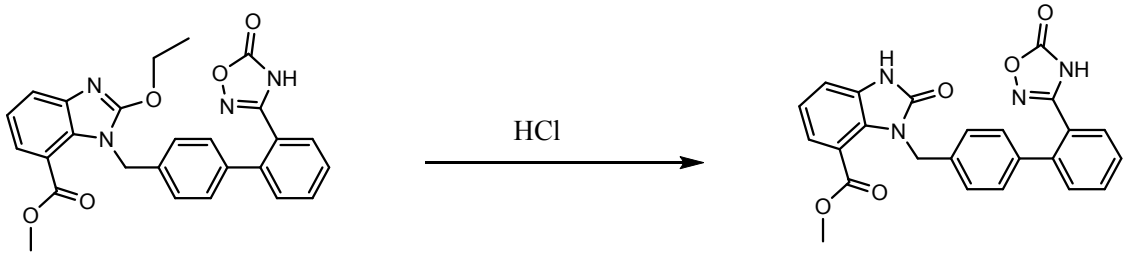
m/z = 444.1798

Impurity I

m/z = 416.1485

Methyl 1-[[2´-(4,5-dihydro-5-oxo-4H-1,2,4-oxadiazol-3-yl) biphenyl-4-yl) Methyl]-2-ethoxy-1H- benzimidazole-7-carboxylate on treatment with conc. hydrochloric acid could result in formation of related substance I.

Pathway for Series 5 *Related substance* (*Related substance* J)



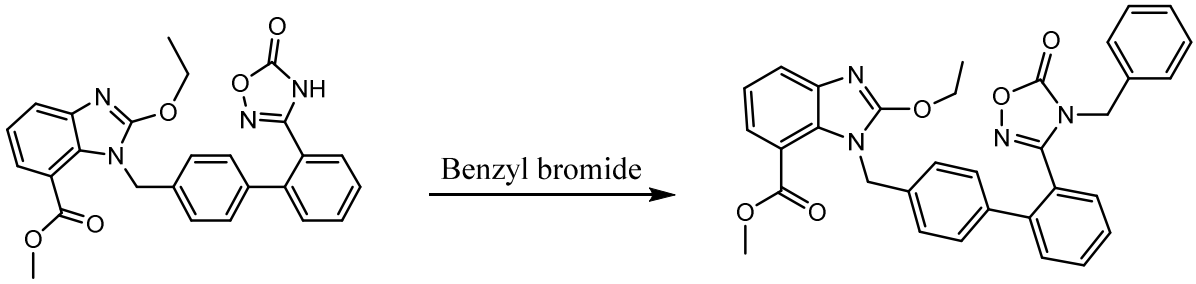
m/z = 470.1590

Impurity J

m/z = 442.1277

Methyl 1-[[2´-(4,5-dihydro-5-oxo-4H-1,2,4-oxadiazol-3-yl) biphenyl-4-yl) Methyl]-2-ethoxy- 1H- benzimidazole-7-carboxylate treated with hydrochloric acid to give related substance J.

Pathway for Series 6 related substance (Related substanceK)



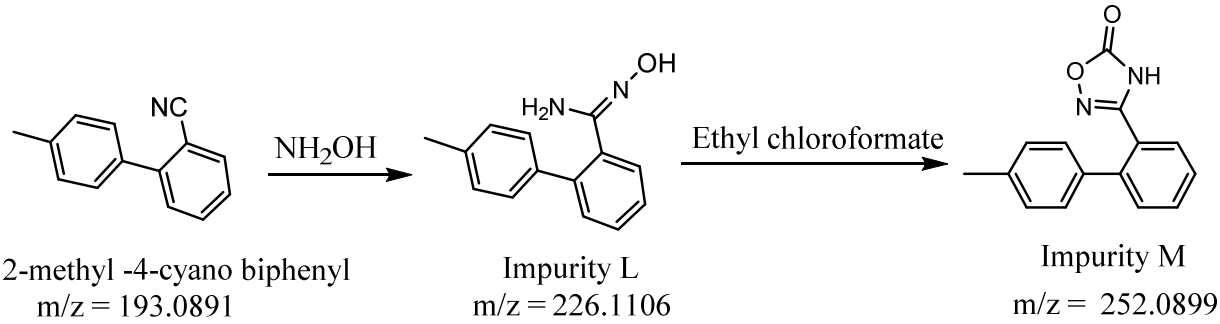
m/z = 470.4850

Impurity K

m/z = 560.6100

Methyl 1-[[2´-(4,5-dihydro-5-oxo-4H-1,2,4-oxadiazol-3-yl) biphenyl-4-yl) Methyl]-2-ethoxy-1H- benzimidazole-7-carboxylate could react with benzyl bromide in presence of a base to give related substance K.

Pathway for Series 7 *Related substance*s (Related substances L and M)



2-methyl -4-cyano biphenyl
m/z = 193.0891

Impurity L
m/z = 226.1106

Impurity M
m/z = 252.0899

2-methyl -4-cyano biphenyl is treated with hydroxyl amine and DMSO to give related substance L which is then treated with ethyl chloroformate to give related substance M.

CONCLUSION

In order to understand the related substance-formation pathway of the antihypertensive drug Azilsartan, knowledge about the different possible related substance and synthetic routes is pre- requisite. Keeping in view the regulatory importance of Azilsartanrelated substances, the process-related related substance in Azilsartan were identified, synthesized, and characterized using mass, infrared (IR), and NMR techniques.In conclusion, total 13 process related substanceof Azilsartan has been synthesized and characterized.

Acknowledgment
We would like to thank Dr. Hari Babu (CEO Mylan Laboratories Ltd India), Mr. Sanjeev Sethi (Head Mylan Global R & D), Dr. Ramesh Dandala (Head MLL R & D), Dr. Suryanarayana Mulukutla (Head Analytical Department MLL R & D) as well as analytical development team of Mylan Laboratories Ltd. for their encouragement and support. We would also like to thankDr Narahari Ambati (Head IPR MLL R & D) & his Intellectual property team for their support.

REFERENCES

[1] M. Gasparo, K. J. Catt, T. Inagami, J.W. Wright, T. Unger, *Pharmacol. Rev.***2000**, 52, 415
[2] W.B. White, M.A. Weber, D. Sica, G.L. Bakris,A. Perez,C. Cao, S. Kupfer, *Hypertension.***2011**, 57, 413.
[3] G.L.Bakris, D.Sica, M.Weber, W.B. White,A. Roberts,A. Perez, C. Cao, S. J. Kupfer, *Clin. Hypertens.***2011**, 13, 81.
[4] D. Sica, W.B. White, M.A. Weber, G.L. Bakris, A. Perez,C. Cao,A. Handley, S. Kupfer, *J. Clin. Hypertens.* **2011**, 13, 467.
[5] H. Rakugi, K. Enya, K. Sugiura, Y. Ikeda, *Hypertens. Res.***2012**, 35, 552.
[6] Y. Kohara, E. Imamiya, K. Kubo, T. Wada, Y. Inada, T. Naka, *Bioorg. Med. Chem. Lett.***1995**, 5, 1903.
[7] T. Naka,Y. Inada, U.S. Patent 5583141 (**1996)**
[8] T. Naka, Y. Inada, Eur Pat. Appl. EP 0520423, (**1992)**
[9] Y. Kohara, K. Kubo,E. Imamiya, T. Wada, Y. Inada, T. Naka, *J. Med. Chem.***1996**, 39, 5228.
[10] T. Kuroita, H. Sakamoto,M.Ojima, U.S. Pat. Appl. 0187269 (**2005)**
[11] T. Kuroita, H. Sakamoto, M. Ojima, U.S. Pat. Appl. 7157584 (**2007)**
[12] S. Radl, J. Cerny,J. Stach, Z. Gablíkova, *Org. Process Res. Dev.***2013**, 17, 77
[13] A. Agarwal, D. Bansal, A.S. Choudhary, S.K. Dubey, H. Mishra, D. Vir, WO 2012107814 A8 (**2012)**
[14] S.D. Dwivedi, K.K. Singh, J.T. Gajera, US 20140113942 A1, (**2014)**
[15] D. Bansal,H. Mishra,S.K. Dubey, A.S. Choudhary, D. Vir, A. Agarwal, A. Daz, US 20130317230 A1 (**2013)**
[16] International Conference of Harmonization (ICH). Q3A(R) Related substance/Impurities in New Drug Substance, Feb. **2002**.